

Award Number: W81XWH-14-1-0441

TITLE: Identifying Determinants of PARP Inhibitor Sensitivity in Ovarian Cancer

PRINCIPAL INVESTIGATOR: Neil Johnson, Ph.D.

CONTRACTING ORGANIZATION:

The Research Institute of Fox Chase Cancer Center
Philadelphia, PA 19111

REPORT DATE: October 2017

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

REPORT DOCUMENTATION PAGE				Form Approved OMB No. 0704-0188	
Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Department of Defense, Washington Headquarters Services, Directorate for Information Operations and Reports (0704-0188), 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to any penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number. PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.					
1. REPORT DATE (DD-MM-YYYY) October 2017		2. REPORT TYPE Annual		3. DATES COVERED 15Sept2016 - 14Sept2017	
4. TITLE AND SUBTITLE Identifying Determinants of PARP Inhibitor Sensitivity in Ovarian Cancer				5a. CONTRACT NUMBER	
				5b. GRANT NUMBER W81XWH-14-1-0441	
				5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S) Neil Johnson, Ph.D. E-Mail: Neil.Johnson@fccc.edu				5d. PROJECT NUMBER	
				5e. TASK NUMBER	
				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) The Research Institute of Fox Chase Cancer Center 333 Cottman Avenue Philadelphia, Pennsylvania 19111 E-Mail: osr@fccc.edu				8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012				10. SPONSOR/MONITOR'S ACRONYM(S)	
				11. SPONSOR/MONITOR'S REPORT NUMBER(S)	
12. DISTRIBUTION / AVAILABILITY STATEMENT Approved for public release; distribution unlimited					
13. SUPPLEMENTARY NOTES					
14. ABSTRACT Cells that are deficient in homologous recombination (HR) DNA repair, such as those lacking functional BRCA1 are highly sensitive to poly(ADP-ribose) polymerase (PARP) inhibitors. Ovarian cancer patients that harbored germ-line <i>BRCA1</i> mutations treated with PARP inhibitors exhibited meaningful responses in early phase clinical trials. However, emerging clinical trial data indicates that PARP inhibitor therapy may benefit only a subset of <i>BRCA1</i> mutation carriers. In this reporting period, we have identified genetic alterations in PARP inhibitor resistant cell lines using whole exome analyses and RNA-seq technologies. We used MDA-MB-436, HCC1395, UWB1.289 and SUM149 cell lines that all harbor BRCA1 mutations to analyze parental PARP inhibitor sensitive and resistant derivatives for gene expression and mutational changes. We identified PHC2, ARID1B, ANKLE1, CHL1 genes that had recurring mutations in PARP inhibitor resistant cell lines. In gene expression analyses, we identified PHC2, ARID1B, ANKLE1, CHL1, BST2, ASNS, EHF genes that had recurring altered expression in PARP inhibitor resistant cells. The next steps of our research will be to use siRNA to alter the expression of the genes identified and determine if they impact PARP inhibitor sensitivity and DNA repair pathways.					
15. SUBJECT TERMS Ovarian cancer, BRCA1, RAD51, PARP inhibitors, platinum, biomarkers, drug resistance					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT UU	18. NUMBER OF PAGES 12	19a. NAME OF RESPONSIBLE PERSON USAMRMC
a. REPORT U	b. ABSTRACT U	c. THIS PAGE U			19b. TELEPHONE NUMBER (Include area code)

Table of Contents

1. Introduction	4
2. Keywords	4
3. Accomplishments	4
4. Impact	8
5. Changes/Problems	8
6. Products	8
7. Participants & Other Collaborating Organizations	9
8. Special Reporting Requirements	10
9. Appendices	10

DOD Annual Report

Principal Investigator: Neil Johnson, Ph.D.
Institution: Institute for Cancer Research
Grant Number: W81XWH-14-1-0441

INTRODUCTION:

Cells that are deficient in homologous recombination (HR) DNA repair, such as those lacking functional BRCA1 are highly sensitive to poly(ADP-ribose) polymerase (PARP) inhibitors. Ovarian cancer patients that harbored germ-line *BRCA1* mutations treated with PARP inhibitors exhibited meaningful responses in early phase clinical trials. However, emerging clinical trial data indicates that PARP inhibitor therapy may benefit only a subset of *BRCA1* mutation carriers. We hypothesize that a range of common ovarian cancer predisposing germ-line *BRCA1* gene mutations produce semi-functional proteins that are capable of providing PARP inhibitor resistance. Our preliminary data suggests that PARP inhibitor selection pressure results in genetic adaptations that enable cells to utilize severely truncated BRCA1 proteins for RAD51 loading and HR repair. Our objectives are to define the BRCA1 peptide region minimally required for PARP inhibitor resistance, and discover genetic alterations that activate DNA end resection as well as mutant *BRCA1* protein stabilization in ovarian carcinomas. The expression of mutant BRCA1 or novel proteins identified to be important for drug resistance will be assessed for their ability to be used as biomarkers of PARP inhibitor or platinum response. *Specific Aims.* 1: Identify the region of mutant BRCA1 protein critical for PARP inhibitor resistance; 2: Identify genetic alterations essential for PARP inhibitor resistance; 3: Determine the ability of identified genetic aberrations to serve as predictive biomarkers. Protein expression in tumors will be assessed for their potential to serve as biomarkers that predict PARP inhibitor or platinum response.

KEYWORDS: Ovarian cancer, BRCA1, RAD51, PARP inhibitors, platinum, biomarkers, drug resistance

ACCOMPLISHMENTS:

Major Task 1: Determine the minimum region of BRCA1 protein required for PARP inhibitor resistance.

Milestone(s) Achieved: Drug resistant cell lines are derived that express different types of mutant *BRCA1* proteins. Target date for completion: 12 months (from start of award). Current status: 100% completed.

Major Task 2: Determine the minimum region of BRCA1 protein required for RAD51 focus formation.

Milestone(s) Achieved: The minimum region of mutant *BRCA1* protein is identified that can contribute to RAD51 loading. Target date for completion: 16 months (from start of award). Current status: 100% completed.

Major Task 3: Generate whole exome sequencing and gene expression data sets for PARP inhibitor sensitive and resistant cell lines.

Milestone(s) Achieved: Identification of genes that are mutated or differentially expressed in PARP inhibitor resistant cells. Target date for completion: 30 months (from start of award). Current status: 100% completed. Completion date: 36 months.

Major Task 4: Validate identified genes as resistance causing through RNAi and cDNA overexpression techniques.

Milestone(s) Achieved: Validation of genes that are mutated or differentially expressed and result in PARP inhibitor resistance. Target date for completion: 36 months (from start of award). Current status: 25% completed. Expected completion date: 44 months.

Major Task 5: Obtain IRB approval for assessing tumor DNA and assembling tumor microarrays.

Milestone(s) Achieved: IRB protocol approved. Target date for completion: 40 months (from start of award). Current status: 100% completed.

Major Task 6: Analyze tumor DNA for mutations.

Milestone(s) Achieved: Identification of gene mutation or protein expression changes in ovarian tumors. Target date for completion: 55 months (from start of award). Current status: 0% completed. Expected completion date: 55 months.

Major Task 7: Determine the ability of potential biomarkers to predict therapeutic outcome.

Milestone(s) Achieved: Biomarkers are ready for application in clinical trials of BRCA1 mutant ovarian cancer and data is published in peer-reviewed journals. Target date for completion: 60 months (from start of award). Current status: 0% completed. Expected completion date: 60 months.

What was accomplished under these goals?

Major Task 3: Generate whole exome sequencing and gene expression data sets for PARP inhibitor sensitive and resistant cell lines.

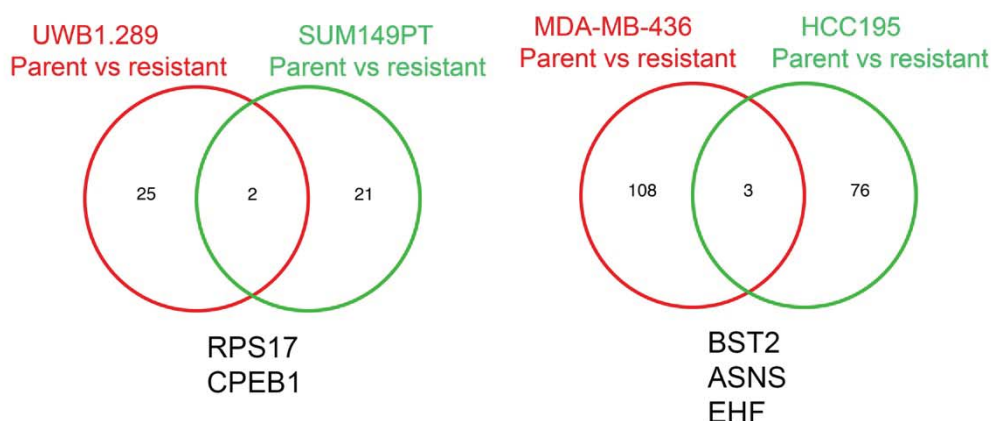
We used MDA-MB-436, HCC1395, UWB1.289 and SUM149 cell lines that all harbor BRCA1 mutations to analyze parental PARP inhibitor sensitive and resistant derivatives for gene expression and mutational changes. To derive PARP inhibitor resistant cells, parental cells were grown in the presence of rucaparib until resistant cells emerged. For exome analyses, we listed genes that were mutated in resistant clones, that were not mutated in the original parental cell line. **Table 1** shows a list of mutations that were detected by exome capture in resistant derivatives of each cell line. Of note, for MDA-MB-436 and HCC1395 cells, we analyzed 2 resistant clones per cell line. Because UWB1.289 and SUM149 cells rapidly acquired PARP inhibitor resistance and the entire population of cells in culture grew through PARP inhibitor treatment (rather than as individual clones where the majority of cells die), we analyzed the entire population of resistant cells for mutational changes.

We expect that genes that are mutated in more than one clone, or more than one cell line, could have importance and contribute to PARP inhibitor resistance. Whereas the majority of mutations acquired are likely bystander mutations that do not directly impact PARP inhibitor sensitivity. Of note PHC2, ARID1B, ANKLE1, CHL1 were selected for follow up functional analyses as mutations were detected in more than one cell line or clone. We are currently beginning to carry out functional analyses, here siRNA is used on resistant cell lines that target these genes and the ability of protein knockdown to re-sensitize resistant cells to PARP inhibitor treatment will be determined. Additionally, mutations could be deleterious to gene function, therefore siRNA will also be used on parental cells to determine if gene knockdown results in resistance.

We have also carried out gene expression analyses using RNA-seq technology to examine global changes in gene expression between parental sensitive and PARPi resistant derivative cell lines (**Figure 1**). To narrow down our list of potential candidate genes that contribute to therapy resistance, we carried out a series of comparisons and analyses. First, we compared each cell parental to PARPi resistant cells to identify genes that had changes in expression in PARP inhibitor resistant cells. Second, we grouped cell lines by the type of BRCA1 mutation and the truncated BRCA1 protein that was generated in the resistant setting. We have previously shown that MDA-MB-436 and HCC1395 cells, each with BRCT domain located BRCA1 mutations, generate BRCT deficient BRCA1 proteins when PARPi resistance occurs. In contrast, UWB1.289 and SUM149 cells both harbor exon 11 located BRCA1 mutations, and when PARPi resistance develops, cells express and exon 11 deficient BRCA1 protein isoform. We therefore examined changes in gene expression that occurred in both MDA-MB-436 and HCC1395 cells, as well as SUM149 and UWB1.289 cells. From these analyses, we found that RPS17 and CPEB1 gene expression were significantly changed in both MDA-MB-436 and HCC1395 PARPi resistant cells. Whereas BST2, ASNS, EHF genes were all altered in both UWB1.289 and SUM149 PARP inhibitor resistant cells (**Figure 1**). The next steps of our research will be to use siRNA to alter the expression of the genes identified and determine if they impact PARPi sensitivity and DNA repair pathways.

Table 1. Genes that are mutated in PARPi resistant cell lines.

MDA-MB-436 clone 1	MDA-MB-436 clone 2	MDA-MB-436 clone 3	MDA-MB-436 clone 4	HCC1395 clone 1	HCC1395 clone 2	UWB1.289	SUM149PT
RNF19B	FMN2	PHC2	RNPC3	ITGB1BP1	PHC2	CRIPAK	PDE4DIP
ZFP69B	TMEM37	LRP1B	REXO2	CHL1	ZFP69B	DSPP	ABCA12
KIAA1239	TMPRSS11A	STAT4	RNFT2	AC092964.1	DDX20	HSPA9	TMEM198
USP53	DND1	HTT	SGPP1	C6orf118	FAM117B	ARID1B	MAGI1
DND1	ARID1B	IL20RA	ANKLE1	C8orf76	AGAP1	NR4A3	NKX1-1
ARID1B	PRKDC	ARID1B	PRR21	KRTAP5-5	CHL1	PAPPA-AS1	HTT
RBM33	MRVI1	ASPH	PPIL4	TRPV4	CAGE1	TTLL11	C5orf30
FAM189A2	RPS6KB2	TFAM	RBM33	POLG	RUNX2	SYT15	CAGE1
GAS1	CASP5	CTNNA3	PRKDC	JUP	FAM184A	ASCL1	EPB41L2
USP31	BCL6B	UBE4A		RNMT	ZNF479	OR5P2	TMEM67
MRPS7	ANKLE1	SPG11		ZNF66	ZNF484	KDM6B	ZNF484
ANKLE1		ANKLE1		ICA1L	NR4A3	ITGA3	KRTAP5-9
ZSCAN5A		ZNF880		MICALCL	ASCL1	SLITRK4	ASCL1
KLHL34		SCRT2			ARHGAP5	ZNF626	GOLGA8A
SAT1		NECAB3			DNAAF1		KRTAP9-1
		DOCK11			TMEM102		NAGLU
					CDRT1		CHMP6
					KRTAP9-1		ZNF432
					MPPE1		ZNF83
					LINGO3		ZNF749
					PLIN4		APOBEC3B
					ZNF44		RPGR
					ZNF791		
					ZNF429		
					ZNF729		
					ZNF726		
					WBP7		
					ZNF610		
					CD93		
					TRIOBP		
					HTATSF1		
					ZNF610		

Figure 1. Venn diagram showing genes that have altered mRNA expression in PARPi resistant cell lines.**What opportunities for training and professional development has the project provided?**

During the third year of this award, I have taken part in the following training activities:

- Discussed progress with Drs. Boyd, Connolly and Golemis.
- Discussed progress with my Chief Scientific Officer Dr. Chernoff.
- Attended Junior Faculty Mentoring Meetings.
- Presented research at the monthly Faculty Seminar.
- Presented research at Molecular Therapeutics Departmental meetings.
- Presented research at FCCC gynecologic oncology meetings.

During the third year of this award, I have taken part in the following professional development activities:

- I frequently attend FCCC Gynecologic Cancer Meetings.
- I attended the AACR advances in ovarian cancer meeting in Pittsburgh.
- I attended the Annual Academy DoD meeting in Pittsburgh.
- I attended Bassett Center for BRCA annual symposium, Philadelphia.
- I was invited to give lectures on my work at Mayo Clinic, Minnesota and Rutgers, NJ.
- I have been awarded an R01 from NCI.

How were the results disseminated to communities of interest?

I have presented my work to FCCC meeting groups described above. Additionally, my student presented a poster on our work and was awarded an AACR-AFLAC travel award to attend the ovarian cancer meeting in Pittsburgh.

What do you plan to do during the next reporting period to accomplish the goals?

We are currently beginning to carry out functional analyses, siRNA will be used on resistant cell lines that target these genes and the ability of protein knockdown to re-sensitize resistant cells to PARP inhibitor treatment will be determined. Additionally, mutations could be deleterious to gene function, therefore siRNA will also be used on parental cells to determine if gene knockdown results in resistance. We will examine the impact of genes on RPA32 and RAD51 foci formation as markers of DNA end resection and RAD51 loading during homologous recombination, respectively.

IMPACT:

We have selected for drug resistant sub-clones by chronically culturing cells in the presence of PARP inhibitor. This has enabled us to identify gene mutations and changes in gene expression that may contribute to HR DNA repair. Our system will be useful for predicting which ovarian tumors will have lasting responses to PARP inhibitor therapy. Furthermore, the genes that we have identified could be developed as biomarkers that predict therapeutic outcome will have value in selecting strategies for clinical management.

What was the impact on the development of the principal discipline(s) of the project?

Nothing to Report.

What was the impact on other disciplines?

Nothing to Report.

What was the impact on technology transfer?

Nothing to Report.

What was the impact on society beyond science and technology?

Nothing to Report.

CHANGES/PROBLEMS:

Changes in approach and reasons for change

Nothing to Report.

Actual or anticipated problems or delays and actions or plans to resolve them

Nothing to Report.

Changes that had a significant impact on expenditures

Nothing to Report.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Nothing to Report.

Significant changes in use or care of human subjects

Nothing to Report.

Significant changes in use or care of vertebrate animals

Nothing to Report.

Significant changes in use of biohazards and/or select agents

Nothing to Report.

PRODUCTS:

Publications, conference papers, and presentations

Nothing to Report.

Journal publications.

Nothing to Report.

Books or other non-periodical, one-time publications.

Nothing to Report.

Other publications, conference papers, and presentations.

Nothing to Report.

Website(s) or other Internet site(s)

Nothing to Report.

Technologies or techniques

Nothing to Report.

Inventions, patent applications, and/or licenses

Nothing to Report.

Other Products

Nothing to Report.

PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS**What individuals have worked on the project?**

Name:	<i>N. Johnson, Ph.D.</i>
Project Role:	Principal Investigator
Researcher Identifier (e.g. ORCID ID):	Neiljohn
Nearest person month worked:	5
Contribution to Project:	Overall administration and guidance of research; Management and training of personnel
Funding Support:	N/A
Name:	<i>J. Boyd, Ph.D.</i>
Project Role:	Mentor
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	1
Contribution to Project:	Dr. Boyd is the mentor on this project. He provides advice, assistance and support.
Funding Support:	N/A
Name:	<i>J. Krais, Ph.D.</i>
Project Role:	Postdoctoral Associate
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	12
Contribution to Project:	Dr. Krais contributed to the cell culture and molecular biology experiments.
Funding Support:	Salary support by NIH T32
Name:	<i>J. Nascon</i>
Project Role:	Graduate Student
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	6
Contribution to Project:	Mr. Nascon assists with the cell culture and molecular biology experiments.
Funding Support:	N/A

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Please see Other Support attached. Changes from the last reporting period are marked with a line in the right hand margin.

What other organizations were involved as partners?

Organization Name: Florida International University

Location of Organization: Miami, Florida

Partner's contribution to the project: Mentor

- Dr. Boyd devotes 10% effort as a mentor on this project. Funds are also allotted for travel.

Organization Name: Temple University

Location of Organization: Philadelphia, PA

Partner's contribution to the project: Consultant

- Dr. Burger provided advice and guidance on the development of biomarkers that predict tumor PARP inhibitor sensitivity. His role will increase in the upcoming years.

Organization Name: University of Washington

Location of Organization: Seattle, WA

Partner's contribution to the project: Consultant

- Dr. Swisher provided advice and guidance on the development of on the biomarkers that predict tumor PARP inhibitor sensitivity. Her role will increase in the upcoming years.

SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: Not Applicable.

QUAD CHARTS: Not Applicable.

APPENDICES

Not Applicable.

Other Support

Johnson, Neil

ACTIVE

W81XWH-14-1-0441 (PI: Johnson)	9/15/2014 - 9/14/2019	40.0%
DOD	\$143,563	4.80 calendar

Identifying Determinants of PARP Inhibitor Sensitivity in Ovarian Cancer

The major goals of this project are: 1) To identify the region of mutant BRCA1 protein critical for PARP inhibitor resistance; 2) To identify genetic alterations essential for PARP inhibitor resistance; and 3) To determine the ability of identified genetic aberrations to serve as predictive biomarkers.

Procuring Contracting/Grants Officer: Kevin Moore, USAMRAA, 820 Chandler Street, Fort Detrick, MD 21702, 301-719-7101

R01 CA214799 (PI: Johnson)	9/12/2017 - 8/31/2022	25.0%
NIH	\$228,750	3.00 calendar

Compensatory Mechanisms that Promote Homologous Recombination in BRCA1 Mutant Cancers

The major goals of this project are to: 1) Investigate the role of BRCA1 mutant alleles in maintaining HR and cancer viability; and 2) Investigate HR-promoting mechanisms contributing to therapy resistance.

Procuring Contracting/Grants Officer: Jason Gill, 9609 Medical Center Drv., BG 9609 RM 2W430, Rockville, MD 20850, 240-276-6885

(PI: Johnson)	5/1/2017 - 4/30/2018	NA
Sandy Rollman	\$50,000	

Investigating Strategies that Prevent PARP Inhibitor Resistance in BRCA1 Mutant Ovarian Cancer

The major goals of this project are: 1) Determine the ability of small molecule inhibitors of resistance pathways to prolong PARPi maintenance therapy in vitro; and 2) Determine the ability of small molecule inhibitors of resistance pathways to prolong PARPi maintenance therapy in vivo.

Procuring Contracting/Grants Officer: Robin Cohen, 210 West Chester Pike, Suite 410, Havertown, PA 19083, 610-446-2272

(PI: Johnson)	4/1/2017 - 3/31/2018	10.0%
Rivkin	\$75,000	1.20 calendar

Investigating the Ability of Splicing Inhibitors to Target BRCA1 Mutant Ovarian Cancer

The major goals of this project are to: 1) Determine the ability of splicing inhibitors to target BRCA1 mutant cancers in vitro; and 2) Determine the ability of splicing inhibitors to target BRCA1 mutant cancers in vivo.

Procuring Contracting/Grants Officer: Kiran Dhillon, PhD., 801 Broadway, Suite 701, Seattle, WA 98122, 206-215-2964

CCRCR17499048 (PI: Johnson)	8/15/2017 - 8/14/2019	25.0%
KOMEN	\$120,000	3.00 calendar

The Ability of Splicing Inhibitors to Target BRCA1 Mutant Breast Cancer

The major goals of this project are to: 1) Determine the ability of splicing inhibitors to target BRCA1 mutant breast cancers in vitro; and 2) Determine the ability of splicing inhibitors to target BRCA1 mutant breast cancers in vivo.

Procuring Contracting/Grants Officer: Susan Fickes, Project Manager, 5005 LBJ Freeway, Suite 250, Dallas, TX 75244, 972-701-2147

COMPLETED

W81XWH-15-1-0197

R21 CA191690

OVERLAP

None

Other Support

Krais, John

Dr. Krais is no longer associated with this project. Dr. Krais' efforts have been replaced by non-key personnel.